(6 months) in normal individuals who are subjected to restricted vitamin A and carotene intake. Therefore, no conclusion can be drawn on our subjects with regard to the effect of mineral oil on the supply of vitamin A as such to the body. Experiments on animals indicate that mineral oil interferes far less with absorption of vitamin A than carotene.[3,5,9,15] Furthermore, mineral oil containing vitamin A provides a ready source of this vitamin to vitamin A-deficient animals.[6]

[15] Rowntree, J. I., *J. Nutr.*, 1931, **3**, 345.

Similar experiments on man are contemplated.

Summary. Continued ingestion of mineral oil as such or in the form of mayonnaise dressing by 20 subjects on a normal unrestricted diet resulted in a moderate decrease in plasma carotene concentration. Vitamin A levels remained unchanged.

It is concluded that simultaneous ingestion of mineral oil with food prevents substantial amounts of food carotene from entering the body.

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Nutritional Requirements of *Tritrichomonas foetus* with Special Reference to Partially Digested Proteins.

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The purpose of this group of experiments is to review the metabolism of the endozoic parasite, *Tritrichomonas foetus,* in order to determine if this organism requires the same general type of nitrogen as that required by other animals, and also to determine the effects of partially-digested proteins on the growth rate of this organism. It is believed that this is the first more or less detailed study of protein nutrition, using synthetic media, in a bacteria-free endozoic protozoan which does not inhabit the blood.

Several methods have been proposed for the cultivation of *Tritrichomonas foetus.* A medium commonly used is egg-serum-Locke's medium (ESL). Collier and Boeck's modification[1] was used in this experiment for the maintenance of the culture. The test tubes used were 4″ x ⅜″ and were filled with 2 cc of liquid medium. After the media tubes had been prepared, they were each inoculated with approximately 380 organisms, this number being obtained from 2 bacteriological loopfuls of a well-mixed, 5- to 7-day-old, ESL culture. All culture tubes were incubated at 25°C in an oven with a saturated atmosphere to prevent evaporation as far as possible. Maximum growth in an ESL culture was obtained in 5 to 7 days.

The method of counting used throughout this experiment is a very simple one. The test tube is shaken thoroughly. Then a bacteriological loopful of liquid is removed aseptically to a clean slide. The number of organisms present in the center of the drop for one ocular field is counted. This procedure is followed 3 consecutive times to insure reasonable accuracy in a minimum amount of time. This method has been calculated statistically to be accurate with a maximum error of ±5%. Two cc of liquid were maintained as the standard volume in all media so that the figures obtained by this counting method in different media would be comparable. Bacteria-free cultures were maintained throughout the experiment.

Three standard media were compared to determine the differences in growth response in dissimilar types of media. Broth-serum-glucose (BSG) described by Andrews and

[1] Collier, J., and Boeck, W. C., *J. Parasit.*, 1926, **12**, 131.

[2] Andrews, J., and von Brand, T., *Am. J. Hyg.*, 1938, **28**, 138.

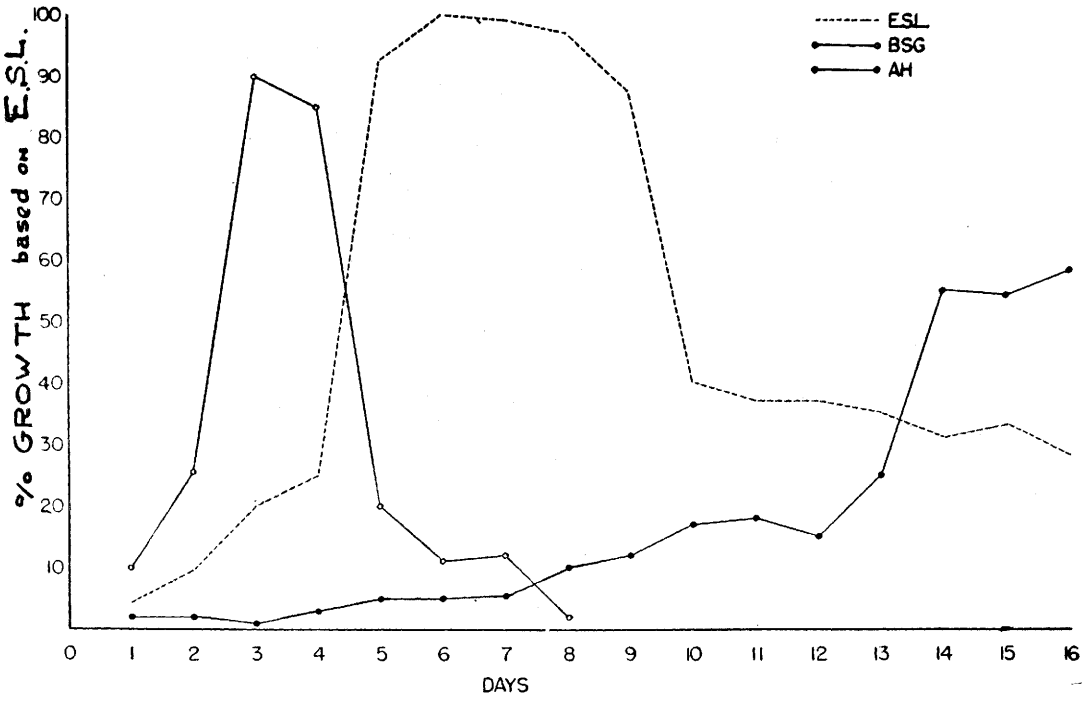

FIG. 1.
Comparison of growth results in Egg-serum-Locke's (ESL), Agar serum (AH), and Broth-serum-glucose media (BSG).

von Brand,[2] agar-serum (AH) used by Weinman[3] and ESL, previously described, were used to represent the 3 types. Fig. 1 shows the comparison between these 3.

Fig. 1 shows that growth in AH is considerably slower than in either of the other media; this probably is due to the high viscosity of the agar. High viscosity is known to inhibit division of organisms.[4] Growth in BSG, it is observed, reaches its peak rapidly and falls in the same manner. A tentative explanation for this in the light of subsequent experiments on digested proteins is that this medium, which is composed of peptones and partially-digested beef proteins, is able to initiate growth better than whole proteins and yet is in some way incomplete.

Emphasis in recent research has been placed on the amino acid requirements of many organisms. The requirements of *Tetrahymena geleii,* a free-living ciliate, have been meticulously worked out by Kidder and Dewey.[5] The same general technics have been used in this analysis of the requirements of *Tritrichomonas foetus.*

The complete amino acid medium of Kidder and Dewey[5] was used in these experiments.

Changes have been made in tryptophane (1— to dl), lysine (dl to 1+), glutamic acid (dl to 1+). In these cases the mg % was varied to insure an equal amount of the active form. The complete medium was supplied from the laboratory of Dr. M. S. Dunn, Department of Chemistry, U.C.L.A. The amino acids were prepared in Dr. Dunn's laboratory. The entire solution was sterilized in amounts of 2 cc per test tube, and the test tubes were then inoculated with a standard ESL culture of 5 days' incubation. Three subsequent transfers were made 5 days apart to be sure that none of the ESL medium carried over in the original transfer was affecting the results.

3 Weinman, D., PROC. SOC. EXP. BIOL. AND MED., 1944, **55**, 82.

4 Hegner, R., and Eskridge, L., *J. Parasit.*, 1936, **22**, 223.

5 Kidder, G. W., and Dewey, V. C., *Physiol. Zool.*, 1945, **18**, 136.

TABLE I.
Growth Results When One Amino Acid Is Omitted from the Complete Amino Acid Medium. Medium does not contain serum.

Amino acid omitted	% growth based on ESL
Alanine	87.84
Arginine	0
Aspartic Acid	84.26
Cystine	83.9
Glutamic Acid	85.7
Glycine	0.6
Histidine	1.08
Hydroxyproline	84.8
Isoleucine	6.0
Leucine	2.4
Lysine	0.6
Methionine	0
Phenylalanine	0
Proline	2.4
Serine	0
Threonine	0.6
Tryptophane	0
Tyrosine	83.8
Valine	1.62
None omitted	85.9

To ascertain the amino acids essential for the growth of *Tritrichomonas foetus*, each amino acid was removed individually from the complete medium. Table I gives the percentage growth based on ESL for each amino acid omitted. Maximum average growth in ESL at 6 days was taken arbitrarily as 100%. These data are based on the 3rd transfer and give the percentage maximum growth obtained regardless of the day on which this was obtained. In the case of those amino acids judged dispensable, the time of maximum growth ranges from the 5th to the 7th day.

The amino acids which can be thus declared essential for the growth of this organism are arginine, glycine, histidine, isoleucine, leucine, lysine, methionine, phenylalanine, proline, serine, threonine, tryptophane, and valine. It will be noted that a negligible amount of growth occurs in the absence of certain essential amino acids; however the number of organisms obtained in these tubes is insignificant according to the counting method used.

There are various possible explanations for this slight growth in the absence of some essential amino acids. Because of the high purity of the amino acids tested, it is doubtful if contamination as reported by other workers[5,6] plays a role here. A more probable explanation is that *Tritrichomonas foetus* is able to carry on a slow synthesis of certain amino acids. This has been shown to occur in comparable experiments with Lactobacilli.[7]

TABLE II.
Comparison of Growth Obtained in the Complete and Basic Amino Acid Media with and without the Inclusion of Serum.

	% growth based on ESL			
	Days			
Medium	4	6	8	10
ESL	25	100	97	40
Comp. A.A.				
—with serum	52	101	101	90
—no serum	50	85.5	81	77.5
Basic A.A.				
—with serum	40	86	85	60
—no serum	40	76	75	61

After determining the amino acids essential to the continued growth of this organism, an attempt was made to establish a vigorous culture on these amino acids alone. Growth in such a medium was 10% lower than that found in the complete amino acid medium. Results such as these have been observed by other workers on different organisms although the cause has not yet been ascertained.[5,8,9] Table II compares growth in the complete and in the basic amino acid media. The improvement in the media on the addition of blood serum indicates that serum may contain an unknown factor acting as a growth stimulant.

Once the required amino acids for *Tritrichomonas foetus* were determined, the growth response of the organism in proteins and in partially-digested protein media was studied. All the protein media were prepared with a sufficient quantity of glucose, vitamins, and other growth stimulants so that any limiting effect would be due for the most part to the protein itself. Previous ex-

[6] Hegsted, D. M., and Wardwell, E. D., *J. Biol. Chem.*, 1944, **153**, 167.

[7] Dunn, M. S., Shankman, S., Camien, M. N., and Block, Harriette, *J. Biol. Chem.*, 1947, **168**, 1.

[8] Hegsted, D. M., *J. Biol. Chem.*, 1944, **156**, 247.

[9] Snell, E. E., and Guirard, B. M., *Proc. Nat. Acad. Sci.*, 1943, **29**, 66.

periments have shown that 0.2% glucose was more than sufficient to satisfy the glucose requirements of *T. foetus*.[2] Although the addition of vitamins to ESL, BSG, or AH in this laboratory had no appreciable effect on growth, vitamins were added to all protein and hydrolysate media in the concentration suggested by Kidder and Dewey.[5] In this experiment the proteins used were casein, lactalbumin, and wheat germ protein. Attempts to use soy bean protein were unsuccessful and gave variable results.

Casein. 1. Vitamin-free casein (Biological Materials Co., Ohio) was mixed with sufficient water to form a paste (approximately 30% protein by analysis). Test tubes were filled with this paste as in the preparation of crumbled egg slants and were overlaid with the standard solution consisting of Locke's medium with added glucose, vitamins, and sterile serum. No growth was obtained, undoubtedly because of the low pH involved. The per cent protein was determined in all cases by a nitrogen analysis on the undiluted protein, determined by the semimicro titration total-nitrogen method.

2. Technical casein was solubilized with NaOH to bring it to a pH of 7. The insoluble casein was filtered off and the supernatant diluted to obtain a 1.5% protein solution by analysis after the addition of the necessary salts, vitamins, and carbohydrates. Some slight growth was obtained.

3. It is believed that drum drying as used in the preparation of technical casein may be detrimental to the protein molecule in a way which may change the biological value of the protein irreversibly. Casein precipitated from skim milk was prepared in the laboratory by the method of Van Slyke and Baker.[10] This casein is relatively ash-free, contains little calcium phosphate, and is readily soluble in dilute alkali. Growth in a 1.5% protein solution of this kind was 10% higher than growth in technical casein solutions at the same pH.

4. Rennin precipitation of milk proteins yields paracasein, a protein sometimes assumed to be the first product in the proteolytic digestion of casein. The paracasein prepared for this experiment by the use of Rennet tablets was not made calcium-free. Growth in this medium was 14% above that obtained in the freshly prepared casein medium.

The preparation of trypsin, pepsin, and papain digests of casein, lactalbumin, and wheat germ protein is shown in Table III. In general the method for preparing the digests is as follows:

The protein solution is made to the desired pH at a concentration of 6% protein. This solution is heated to the required temperature and the necessary amount of enzyme added. Since it was considered undesirable to add a preservative such as toluene during the digestion, the equipment used and most of the materials were first sterilized to keep down as much as possible bacterial contaminants. After the digestion had been carried to the desired stage, the enzyme was inactivated by autoclaving at 15 lb pressure for 5 minutes. Following inactivation, the pH of the digest was raised to pH 7.7 with rapid stirring to dissolve as much of the protein precipitated during the digestion as possible. The digest was then filtered through loose cotton to remove the undissolved protein. As far as can be ascertained the only amino acid precipitate forming after this procedure is tyrosine, a dispensable amino acid for *Tritrichomonas foetus*.

The digest was then made into a medium containing 1.5% of the protein digest, vitamins, glucose, salts, and human blood serum. Two cc of this complete medium were used for each test tube (4″ x ⅜″) and sterilized in the usual manner. Much better growth was obtained in media with the addition of serum. In some cases no growth was observed without serum; in others, although growth was obtained, the amount was insignificant. This stimulatory action of added serum had been noted also in the amino acid media.

Lactalbumin was precipitated from whey at pH 6.0 and then redissolved after wash-

[10] Van Slyke, L. L., and Baker, J. C., *J. Biol. Chem.*, 1918, **35**, 127.

TABLE III.
Preparation and Growth Results in Proteins and Protein Digests.

Substrate	Enzyme	Subs. conc. %	Enzyme conc.	pH	Temp., °C	Hr	% total nitrogen in sol'n	Day of max. growth	% growth based on ESL
Casein—acid precipitated whole protein—								5	27
Casein	Trypsin	6	2%	8	45	6	48.8	7	105
,,	,,	6	1% added every 12 hr until 5%	8	45	72	90.0	6	75
,,	Pepsin	6	Same as above	6	45	72	87.6	7	85
,,	Papain	6	1%	6	45	25	8.7	7	35
Lactalbumin—freshly precipitated whole protein—								6	33
,,	Trypsin	2	2%	8	50	8	53.0	8	102
Lactalbumin	,,	2	1% added every 12 hr until 5%	8	50	48	91.7	5	82
Wheat Germ Protein—freshly precipitated whole protein—								6	20
WG Protein	Trypsin	6	2%	8	50	3	29.7	6	86
,, ,,	,,	6	2%	8	50	6	51.8	5	102
,, ,,	,,	6	2%	8	50	12	67.7	7	98
,, ,,	,,	6	1% added every 12 hr until 5%	8	50	48	83.6	5	69

ing at pH 7.0. Sufficient lactalbumin was not available to make a complete series of digests.

Wheat germ protein was prepared from the whole germ by suspending it in dilute alkali. The soluble protein was then freed from the salts and carbohydrates by acid precipitation. The washed protein was solubilized at pH 7. Growth results in protein and protein digests of casein, lactalbumin, and wheat germ protein are shown in Table III.

Proteins are thought to be differentiated on the basis of the amino acid grouping present within themselves. These groupings are said to make each protein different in structure and in biological value. The proteins used in this experiment are of varying B.V.; the order of high to low is lactalbumin, casein, and wheat germ protein. (Data on biological values were obtained at the Protein Conference at Rutgers University, N. J., 1945). Although the method of measuring B.V. in the protozoan may not be the same as in the metazoan, the value of the protein as a source of certain amino acids can be ascertained. From the results with *Tritrichomonas foetus,* a similar B.V. order is indicated (Table III).

The results obtained in enzyme digests of these proteins can be stated thus: *Tritrichomonas foetus* requires not only the presence of certain amino acids for good growth but in addition responds better to proteins digested to some extent. The response to whole proteins is very low, unmeasurable without the addition of serum. As the protein is digested more and more and in various ways, as the amount of free amino acid as compared with the amount of soluble whole protein becomes greater and greater, as the time of digestion becomes longer, the growth response becomes better until a certain degree of digestion is attained. It will be observed that basically the results are similar regardless of the protein or enzyme used.

The decrease in the maximum growth rate as the digestion of the proteins approaches the amino acid stage indicates perhaps that *Tritrichomonas foetus* needs strepogenin as

well as amino acids for normal growth. Woolley[11-13] has demonstrated the role of strepogenin for normal growth in bacteria, rats, mice and chicks.

Summary. 1. The amino acids essential for the growth of *Tritrichomonas foetus* were determined in a medium of known concentration. This flagellate utilizes nitrogen in approximately the same way as do higher organisms. The amino acids essential for *T. foetus* were determined to be arginine, glycine, tryptophane, histidine, isoleucine, leucine, lysine, threonine, methionine, phenylalanine, proline, serine, and valine.

2. Serum contains some factor capable of improving the media tested.

3. Casein, lactalbumin and wheat germ protein were tested for their growth properties. Results indicate the possible use of *T. foetus* to determine the biological values of proteins.

4. With whole protein, growth is relatively low. In digests, growth improves until the percentage of soluble nitrogen reaches 50%. As digestion continues and more nitrogen goes into solution, the growth rate decreases. This decrease may be due to destruction of strepogenin.

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11 Sprince, H., and Woolley, D. W., *J. Am. Chem. Soc.*, 1945, **67**, 1734.

12 Woolley, D. W., *J. Biol. Chem.*, 1945, **159**, 753.

13 Woolley, D. W., *J. Biol. Chem.*, 1946, **162**, 383.

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Effects of Various Sex Hormones on Excretion of Pregnandiol Early in Pregnancy.*

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Smith and Smith[1] reported that the administration of diethylstilbestrol to a patient during pregnancy would increase the excretion of pregnandiol in the urine. They concluded that synthetic estrogens will stimulate steroid metabolism in the placenta increasing the amount of available progesterone. These authors suggested the use of increasing amounts of diethylstilbestrol to prevent the accidents of late pregnancy, beginning with 30 mg per day at the 16th week of the gestation and increasing the daily dose 5 mg each week until the 35th week. Furthermore, it has been recommended by others that large amounts of steroid hormones be administered early in pregnancy to prevent abortion and to treat threatened abortion.

The purpose of the present study is 2-fold: (1) to determine if the sex hormones will increase the production of progesterone by the placenta early in pregnancy and, (2) to study human fetuses from mothers who have received large amounts of these substances in order to establish that no untoward changes have resulted. The second phase of this study is extremely important in view of the changes induced in the reproductive organs of litters of experimental animals by the ad-

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1 Smith, O. W., Smith, G. V. S., and Hurwitz, D., *Am. J. Obst. and Gynec.*, 1946, **51**, 411.